

# Monitoring cerebral oxygenation and local field potential with a variation of isoflurane concentration in a rat model

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**Abstract:** We aimed to investigate experimentally how anesthetic levels affect cerebral metabolism measured by near-infrared spectroscopy (NIRS) and to identify a robust marker among NIRS parameters to discriminate various stages of anesthetic depth in rats under isoflurane anesthesia. In order to record the hemodynamic changes and local field potential (LFP) in the brain, fiber-optic cannulae and custom-made microelectrodes were implanted in the frontal cortex of the skull. The NIRS and LFP signals were continuously monitored before, during and after isoflurane anesthesia. As isoflurane concentration is reduced, the level of oxyhemoglobin and total hemoglobin concentrations of the frontal cortex decreased gradually, while deoxyhemoglobin increased. The reflectance ratio between 730nm and 850nm and burst suppression ratio (BSR) correspond similarly with the change of oxyhemoglobin during the variation of isoflurane concentration. These results suggest that NIRS signals in addition to EEG may provide a possibility of developing a new anesthetic depth index.

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## 1. Introduction

In clinical practice, estimating the depth of anesthesia (DOA) is important for monitoring safety during surgical treatment. Light anesthesia may cause intraoperative awareness leading to negative psychological effects and consequent medico-legal problems [1]. Deep level of anesthesia can affect the hemodynamic status during surgery and recovery. Since the brain is the effect-site of anesthetic drugs, commercially available anesthetic depth indicators employ different algorithms based on the analysis of spectral changes in electroencephalogram (EEG) [2]. However, this may give rise to erroneous DOA estimates in patients with abnormal EEG [3]. For instance, the bispectral index (BIS), a widely used anesthetic depth monitor, has been reported to underestimate or overestimate anesthetic depth in many clinical scenarios [3].

Anesthetic drugs inhibit neuronal activity in the brain and affect cerebral metabolism [4–6]. Brain oxygenation levels can be monitored to evaluate cerebral metabolism using near-infrared spectroscopy (NIRS) [7,8]. NIRS is a widely used noninvasive optical technique that allows the measurement of hemoglobin oxygenation to evaluate oxygen consumption within living tissue based on the differences in near-infrared (NIR) absorption [9–11]. It can be used to measure the changes of oxyhemoglobin (OHb) and deoxyhemoglobin (RHb) concentration and total blood volume (sum of OHb and RHb) in intact tissues and has been applied to breast cancer detection [12], brain functional study [13] and critical care research [14]. In addition, NIRS is noninvasive, stable from electronic artifacts, cheaper, simple to make and compatible with other modalities such as EEG [15]. In this study, we aim to identify a robust marker to discriminate DOA with NIRS in rats under isoflurane anesthesia.

## 2. Experimental methods

### 2.1 Animal care and use

The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Gwangju Institute of Science and Technology. Eight adult male Long Evans rats weighing 370–610 g were used for this study. The rats were housed at constant room temperature ( $24 \pm 1^\circ\text{C}$ ) maintained on a day-night cycle (lights on at 9:00 AM, and off at 6:00 PM) with free access to water. All experiments were performed during daytime.

### 2.2 Surgical implantation of electrodes and fiber-optic cannulae

Custom-made epidural screw electrodes and fiber-optic cannulae were implanted in the frontal skull of each rat for recording blood oxygenation level changes and the local field potential (LFP) in the brain. Before the surgical implantation, the rat was anesthetized with a mixture of ketamine hydrochloride ( $100 \text{ mg kg}^{-1}$ ) and xylazine ( $7 \text{ mg kg}^{-1}$ ). The rat was then moved to a stereotaxic frame and anesthesia was maintained by 1.5% isoflurane administered through a nasal cone. We made an incision of  $\sim 2 \text{ cm}$  of scalp along the midline and the pericranium was removed to expose the skull. A durotomy was followed by making small holes in the skull. Through the holes, we implanted electrodes and fiber-optic cannulae with the use of a stereotaxic frame. Electrodes were placed 5.5 mm in the anterior of the bregma and  $\pm 3.0 \text{ mm}$  lateral from the midline. Fiber-optic cannulae were placed 2.5 mm in the anterior of the bregma and  $\pm 2.5 \text{ mm}$  lateral from the midline. The implantation was completed by securing the electrodes and fiber-optic cannulae on top of the skull using dental cement. Rats were then transferred to a recovery box.

### 2.3 Experimental protocol

The experiment was performed at least 4 days after the implantation of fiber-optic cannulae and electrodes to allow rats to recover fully from postoperative complications such as hemorrhage, shock, pain and infection from surgery before performing the experiments. The optical fibers in the implanted fiber-optic cannulae were connected to the light source (left

cannula) and CCD spectrometer (right cannula) through optical patch cables. The implanted electrodes were connected to a custom-made LFP recording device. Prior to obtaining NIRS signals and LFP under isoflurane anesthesia, both signals were recorded from freely moving rats (awake state). We considered the awake state signals as the individual baseline reference. After acquiring a baseline reference data for 30 minutes, signals were obtained at various levels of anesthesia with a protocol shown as below.

Isoflurane anesthesia was introduced with 50% oxygen through a nasal cone. Initially, 3.0% isoflurane was administered to induce anesthesia for 3 minutes. Isoflurane concentration was then decreased to 2.5% and administered for 7 minutes. The initial 2 minutes was to allow the blood plasma and the brain to reach pseudo state equilibrium. The DOA was evaluated for each isoflurane concentration administered constantly from 2.5% and decreased by 0.5% after every 5 minutes. After administering 1.0% isoflurane for 5 minutes, the anesthesia was discontinued and we waited to detect the first movement caused by awakening from the anesthesia state. A gas analyzer (B40 model, GE Healthcare) was used to monitor the inhalational oxygen percentage, isoflurane concentration, and end-tidal CO<sub>2</sub> during the measurement. The NIRS signals and LFP were simultaneously and continuously recorded from the awake state until 30 minutes after the first movement (recovery state). At the end of each experiment, the rat was allowed to fully recover from anesthesia.

#### 2.4 Continuous-wave near-infrared spectroscopy (CWNIRS)

For this study, a continuous-wave near-infrared spectroscopy (CWNIRS) system was used to measure the change of oxy-(OHb), deoxy-(RHb), and total hemoglobin (THb) concentration in the brain. The CWNIRS system consists of a tungsten-halogen light source (HL-2000-HP, Ocean Optics Inc.) and a 16-bit CCD spectrometer (USB4000, Ocean Optics Inc.) as detector [16]. Light was delivered to the left frontal lobe via a home-made fiber optic cannula with an optical fiber (Thorlabs FT400EMT, 0.39 NA, Ø400 µm Core) and the diffuse reflectance was measured from the right frontal lobe through another home-made fiber optic cannula. The separation between the light source and detector cannula was 5 mm.

#### 2.5 NIRS signals process

The NIR spectra were acquired at a frequency of 3 Hz and intensity values of five wavelengths (730, 750, 800, 830 and 850 nm) were recorded on a computer to calculate the changes of OHb, RHb and THb concentration. If we assume that the absorbance of OHb and RHb dominates the attenuation of the NIR range and also scattering is constant, the change in [RHb] and [OHb] can be obtained based on the modified Beer-Lambert's law [17,18].

$$\begin{pmatrix} \Delta[RHb] \\ \Delta[OHb] \end{pmatrix} = \frac{1}{d \times DPF} \begin{pmatrix} \epsilon_{RHb}^{730} & \epsilon_{OHb}^{730} \\ \epsilon_{RHb}^{750} & \epsilon_{OHb}^{750} \\ \epsilon_{RHb}^{800} & \epsilon_{OHb}^{800} \\ \epsilon_{RHb}^{830} & \epsilon_{OHb}^{830} \\ \epsilon_{RHb}^{850} & \epsilon_{OHb}^{850} \end{pmatrix}^{-1} \begin{pmatrix} \Delta OD^{730} \\ \Delta OD^{750} \\ \Delta OD^{800} \\ \Delta OD^{830} \\ \Delta OD^{850} \end{pmatrix} \quad (1)$$

Where  $\Delta OD^\lambda$  is the change of optical density at wavelength  $\lambda$ ,  $\epsilon_{RHb}^\lambda$  and  $\epsilon_{OHb}^\lambda$  are the extinction coefficients of RHb and OHb at wavelength  $\lambda$  respectively [19].

The path length is determined by  $d \times DPF$ ,  $d$  is the source-detector separation distance and  $DPF$  is the differential path length factor [20]. The change in total hemoglobin concentration ( $\Delta[THb]$ ) can be obtained by taking the sum of  $\Delta[RHb]$  and  $\Delta[OHb]$ .

To reduce an effect of cerebral blood volume change (CBV) during anesthesia on NIRS signals, we calculated a ratio of reflectance intensity (RRI) between 730 nm and 850 nm by

dividing the reflectance of both 730 nm (R730) and 850 nm (R850) by 800 nm (R800) in which RHb and OHb have identical extinction coefficient values.

$$RRI = \frac{R_{730} / R_{800}}{R_{850} / R_{800}} \quad (2)$$

## 2.6 Statistical analysis of NIRS signals

NIRS signal measurements were recorded from each rat (N=8) during the experiment. For the statistical analysis of NIRS parameters, we selected the last 1 minute data from 5 minutes of each four different isoflurane concentration, 5 minutes data in awake state (20~25 minutes from start point of awake state) and recovery state (7~12 minutes from first spontaneous movement of subject including eye blinking and whisker movement). We then calculated the mean value with the standard deviation from each data. Two-tailed paired t-test was performed to compare the [OHb], [RHb], [THb] and RRI among the awake state, the different anesthetic levels, and the recovery state after identifying the normality of NIRS data using Shapiro–Wilk test. If the null-hypothesis of this test is rejected, Wilcoxon-rank sum test was conducted to investigate the statistical differences of NIRS signals related to isoflurane concentration.

## 2.7 LFP signals process

To compare the performance of NIRS signals as an anesthetic depth indicator, we obtained LFP to identify burst suppression, a validated index of anesthetic depth [21]. After the surgery of microelectrodes implantation, we connected microelectrodes to the custom made LFP recording device. LFPs like EEG are easily contaminated by external noises generated by various external factors. To remove noises in LFPs at each targeted areas, we applied notch filtering to reject the 60Hz line noise. LFPs data were preprocessed and analyzed using custom made analysis software. To visually compare the change of burst suppression lightening on anesthetic levels, we selected representative artifact-free 30 sec LFP epoch from four different anesthetic levels, awake state and recovery state (Fig. 2).

In order to quantify burst suppression, we calculated burst suppression ratio (BSR) which is defined as the fraction of time spent in suppression per epoch. One-minute data from each isoflurane concentration and five-minute data from awake and recovery state were selected as each epoch same as NIRS parameters analysis shown in the previous section. All the raw data were filtered between 0.5 and 32 Hz which covers the most of cerebral signals before the BSR calculation [22]. If there is no presence of suppression during an epoch, then BSR is “0” while BSR will be “1” when suppression exists throughout the duration of an epoch.

In this study, a suppression period was defined as follows: a suppression period starts when LFP amplitudes remain below the threshold value for more than 0.5 s. The suppression period lasts as long as amplitudes remain below the threshold value. The time point when amplitude rises above the threshold was defined as the end of suppression period. The threshold value was manually set to be 33% of standard deviation value from awake state for each rat (Fig. 5).

## 3. Results and discussion

The [OHb] and [THb] increased sharply during the anesthetic induction at 3.0% of isoflurane, while [RHb] decreased. After the anesthetic induction, [OHb] and [THb] decreased gradually with the reduction of isoflurane concentration, while [RHb] increased until 1.5% of isoflurane. However, some rats exhibited an abrupt increase of [OHb] and [THb], and decrease of [RHb] during 1.0% isoflurane administration.

Depending on the presence or absence of this phenomenon, we divided the results into two groups with either ‘awaken during anesthesia (AA)’, characterized by the presence of abrupt increase of [OHb] and [THb]; or ‘not awaken during anesthesia (NAA)’, characterized



by the absence of abrupt increase of [OHb] and [THb]. Figure 1 (A) and (B) show the change of [OHb], [RHb] and [THb] from a representative of each group.

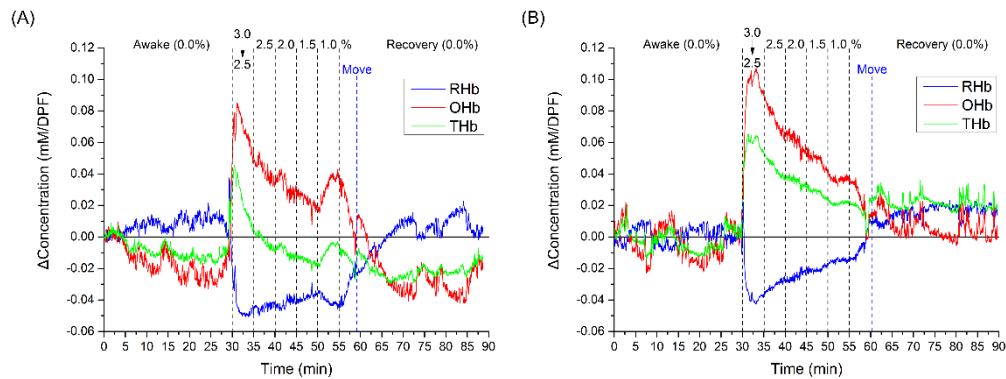


Fig. 1. The representative changes of [OHb], [RHb] and [THb] before, during and after anesthesia from AA and NAA groups. Depending on the existence of rapid increase in the [OHb] level change during the administration of 1.0% of isoflurane, the results are divided into 'AA' group (n=4) (A) and 'NAA' group (n=4) (B).

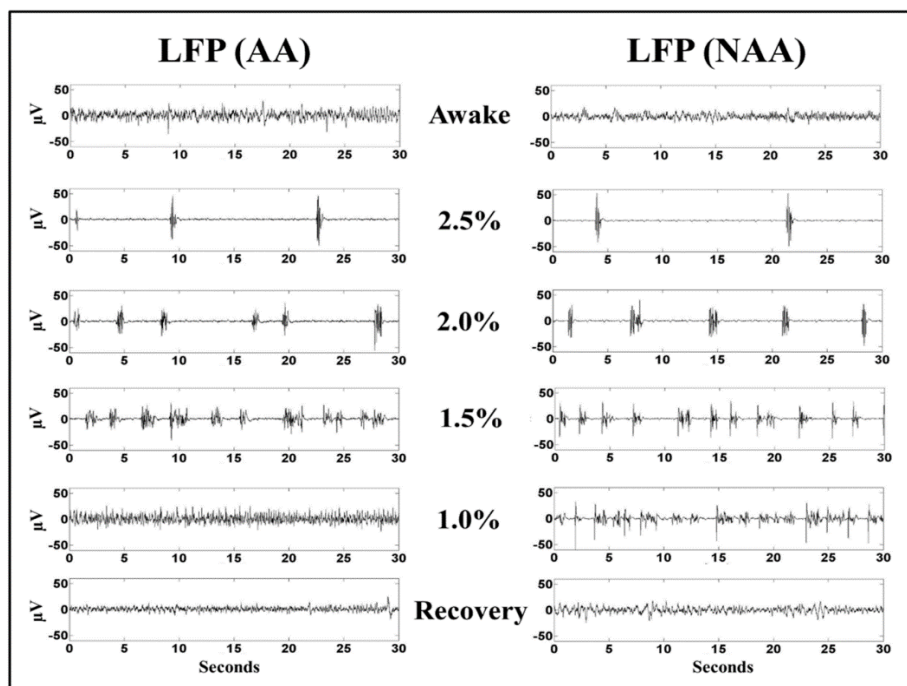


Fig. 2. The representative changes of LFP before, during and after anesthesia from 'AA' and 'NAA' groups. Since spectral changes in the two bipolar montages were almost identical, only the results of left frontal electrode are reported here. Numbers represent the percentage of isoflurane.

In both groups, the LFP consists of low amplitude and high frequency during the awake and recovery state (Fig. 2). On the other hand, a burst suppression pattern was observed while 2.5%, 2.0% and 1.5% of the isoflurane was administered. The duration of burst suppression was reduced as the anesthetic concentration went down. At 1.0% isoflurane concentration, different results were obtained from the 'AA' and 'NAA' group. In the 'AA' group, the burst

suppression was not observed and the LFP pattern is similar to the awake state. In the 'NAA' group, the burst suppression was observed though the duration time of burst suppression was reduced compared to higher isoflurane concentrations.

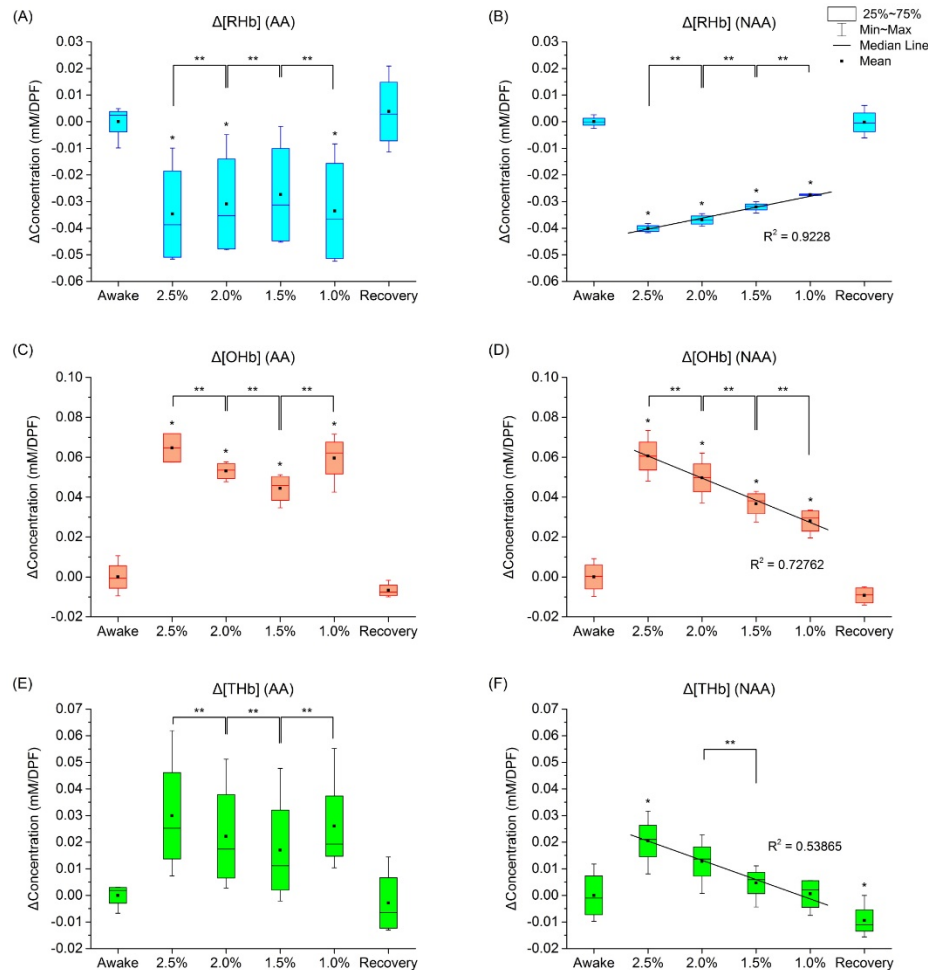


Fig. 3. The changes of [OHb], [THb], and [RHb] for varying isoflurane concentration from 'AA' and 'NAA' groups. The asterisk indicates p value less than 0.05 compared to awake state and the double asterisk indicates p value less than 0.05 compared to each other. The results of simple linear regression analysis are shown on the left column for the NAA group.

[OHb], [RHb], and [THb] values were compared between the awake state, the states during different isoflurane concentrations and the recovery state which are shown in Fig. 3. [OHb] and [THb] were significantly increased ( $P < 0.05$ ), while [RHb] were significantly decreased ( $P < 0.05$ ) during isoflurane anesthesia compared to the awake state. The changes of [OHb], [RHb] and [THb] corresponded linearly to the change in isoflurane concentration in NAA group while AA group showed a reversal of each parameter at 1.0% isoflurane. We also conducted a simple linear regression analysis of the data on anesthetic state for the NAA group to see how they correspond linearly to the change of isoflurane concentration (Fig. 3).

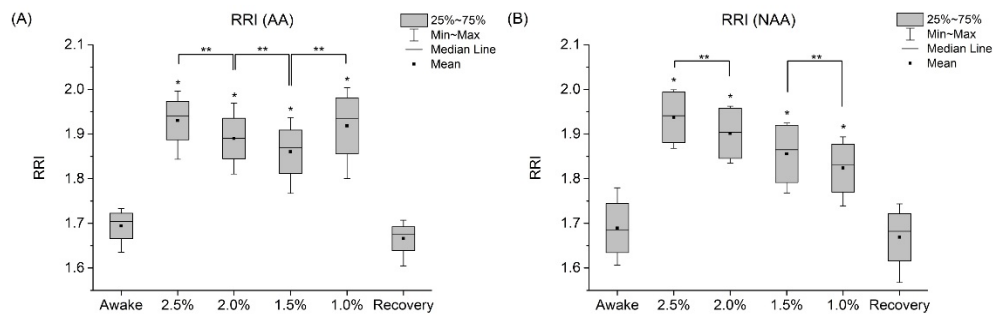


Fig. 4. The changes of RRI value depending on the isoflurane concentration from 'AA' and 'NAA' groups. The asterisk indicates p value less than 0.05 compared to awake state and the double asterisk indicates p value less than 0.05 compared to each other.

The RRI values which are not affected by blood volume change during anesthesia were also compared between the different states (Fig. 4). Overall, RRI followed a similar trend of [OHb] (Fig. 3c and 3d) from both AA and NAA group.

Figure 5 illustrates the change of BSR compared with RRI of all eight subjects. In NAA group, the change of BSR value followed a similar tendency of RRI. On the other hand, AA group showed a different trend between BSR and RRI from 1.5% to recovery state. Unlike NAA group, BSR value at 1% isoflurane from all the subjects are close to the values of awake state which indicates the awakening from anesthesia. However, RRI showed the opposite trend and increased from the values at 1.5% isoflurane.

The technique of NIRS enables the noninvasive monitoring of the hemodynamic changes related to cortical activity [23]. For example, increasing task load gives rise to an increase in blood oxygenation as evaluated by NIRS in the frontal area involved in attention and cognitive perception [24]. In this sense, the technique facilitates the understanding of brain activation by providing markers associated with cerebral metabolism. Most of general anesthetics inhibit cerebral metabolism depending on the dose [25]. Therefore, cerebral oxygen utilization is expected to decrease proportionally with anesthetic depth while inducing a rise in [OHb] and a decline in [RHb] as anesthetic depth increases. Consistent with this hypothesis, we have shown that [OHb] and [THb] decreased gradually with the reduction of isoflurane concentration, while [RHb] increased. This observation corresponds similarly to changes in LFP burst suppression patterns. To the best of our knowledge, this is the first study to identify a potential NIRS-based parameter to reflect anesthetic depth. Considering that NIRS signals proportionally changed in accordance to the change in different anesthetic levels, our results suggest that NIRS signals in addition to EEG have the potential to be a new anesthetic depth index.

It is well known that alveolar concentration of anesthetic agent is a surrogate parameter of the concentration of anesthetic gas on the brain [26,27] and alveolar concentration is equal to the expiratory anesthetic gas concentration which can be monitored by using gas analyzer. The accuracy of the methods were proven and became standard of anesthetic care and monitoring [28].



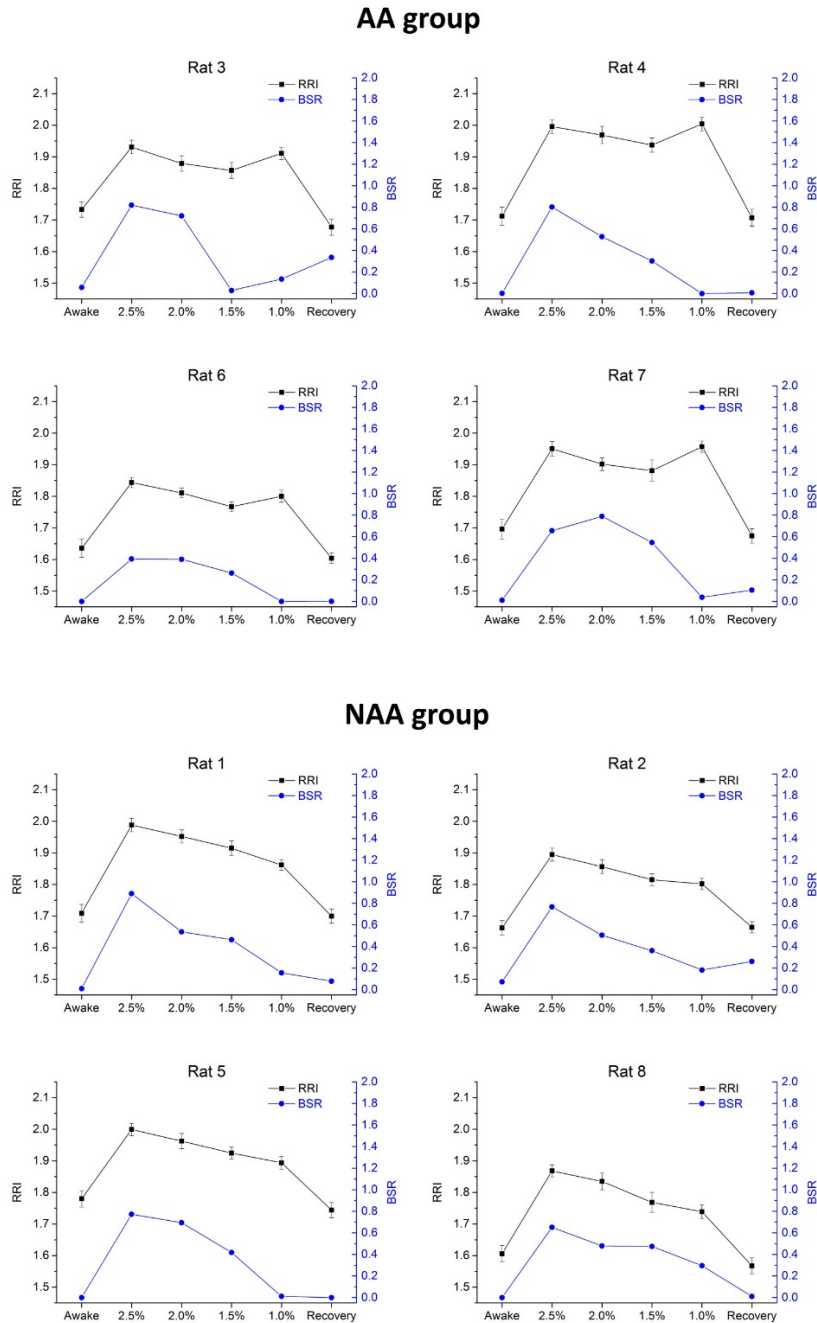


Fig. 5. The change of RRI value and BSR of all eight subjects.

In our experiments, we used GE healthcare's B40 monitor and also applied customized rat mask to minimize the dead space of respiratory circuit. Empirically, we found that the expiratory isoflurane gas concentration became equal to the set value of isoflurane vaporizer after 20~30 seconds of adjustment. In other words, it only takes 20~30 seconds for the brain to reach the isoflurane concentration which we set using the vaporizer. Empirically, we found that hemodynamic changes started to become stable in 5 minutes when isoflurane

concentration was reduced by 0.5%. Since the isoflurane has higher partition coefficients on rat's blood and fat [29], prolonged duration of anesthesia can lead to the accumulation of isoflurane in the rat brain. Therefore, we decided that a five-minute interval would be long enough for the rat's alveolar concentration to reach the target concentration and short enough to prevent isoflurane accumulation in brain.

In this study, we obtained NIRS signals in the frontal brain areas since it is closely related to cognitive perception and recognized as one of the potential site of anesthetic mechanisms [30,31]. In clinical practice, the frontal area is easily accessible to obtain bio-signals in the brain, making this region the popular site for sensor attachment. The BIS, the most widely used anesthetic depth monitor, utilizes EEG signals to calculate anesthetic index at frontal area obtained from sensors attached to the forehead. Therefore, we selected the frontal brain area to monitor NIRS signals.

In order to obtain signals from the brain, we used invasive measurement of NIRS and LFP from implanted electrodes and fiber-optic cannulae. This invasive measurement can minimize the motion artifacts and signal contamination from the skull and scalp since the signals are obtained directly from the brain. In this regard, it is expected that the results of this study can be used as a reference for a non-invasive method in future research.

Cerebral blood volume (CBV) can be affected by hypercapnia due to respiratory depression during anesthesia [32]. In clinical practice, a ventilator is generally used to prevent respiratory depression and maintain the normal breathing condition of the patient during anesthesia. However, in this study, the subjects were allowed to breath spontaneously without a ventilator. Therefore, it will be important to consider the effect of CBV change on NIRS signals. To minimize the potential effect of CBV change on NIRS signals, we presented reflectance ratio value (RRI) as a new NIRS parameter. The RRI is not affected by CBV change since the reflectance of 730nm and 850nm was normalized by the reflectance of 800nm which is the isosbestic point of OHb and RHb. The trend of RRI value during anesthesia was similar to that of [OHb] from both AA and NAA group, suggesting that this parameter reflects the change of cerebral oxygen metabolism associated with anesthesia irrespective of the modes of ventilation.

To evaluate certain inhalation anesthetic's effect on brain, minimum alveolar concentration (MAC) is a useful parameter. The MAC of an inhaled anesthetic is the alveolar concentration that prevents movement in 50% of patients in response to a noxious stimulus such as skin incision. MAC allows comparisons of potency between agents, and provides a standard for experimental evaluations. In clinics, concentration of inhalation anesthetic agent should be maintained 1 MAC during surgery [33]. The 1 MAC value of isoflurane for rat is about 1.5%. So, we could consider that 2.5% (1.67 MAC, nearly comparable to MAC-BAR (1.7~2.0 MAC) which is defined as the concentration required to block autonomic reflexes to noxious stimuli) of isoflurane is a high enough concentration attenuating autonomic responses completely from noxious stimuli for rat and 1.0% (0.67 MAC) is low enough to observe cerebral hemodynamics change during insufficient anesthetic condition [34]. Therefore, 1.0~2.5% of isoflurane can be considered as clinically relevant concentrations.

Since 1% of isoflurane concentration is corresponding to 0.67 MAC, there is a high chance of rat to awake from anesthesia (AA group). The abrupt increase of [OHb] and [THb] at 1% isoflurane from AA group can be explained as follows. To recover from anesthesia, disorganized neuronal connections should be restored, thereby, increasing the possibility of neurovascular coupling [35]. This inevitably leads to an increase in cerebral blood flow which will increase [OHb], [THb] and also the RRI value. In AA group, BSR values at 1% isoflurane also became close to the values of awake state which supports our explanation of sudden changes of NIRS parameters at 1% isoflurane.

This is clinically meaningful since the sudden increases of [OHb], [THb], and RRI can be an indicator that awakening from anesthesia is imminent. Indeed, rats in AA group exhibited spontaneous movement within a few minutes after the observation of abrupt changes in NIRS

signals. This suggests that NIRS signals can be used as anesthetic depth indicators as well as predictors of awakening during general anesthesia. Integrating between various brain regions after discontinuation of anesthetics is necessary for recovery from anesthesia, requiring significant changes in neurovascular coupling and cerebral metabolism. In this sense, the current technique may give insight to elucidate the mechanism of recovery of consciousness from anesthesia and its long-term effect.

However, we note some limitations in this study. The effect on cerebral metabolism may be different depending on the type of inhalational or intravenous anesthetics [36]. Therefore, a further study is required to identify the usefulness of NIRS signals as an indicator of DOA depending on the types of anesthetics. Also, in this study, the quantitative relationship between anesthetic depth and changes of NIRS indicator could not be obtained from this result though we have found that NIRS signals proportionally changed with anesthetic levels. Direct pharmacodynamics relationship between inhaled anesthetics and NIRS signal including RRI can be investigated in a quantitative way, thereby monitoring DOA directly with NIRS. NIRS does not show the DOA information directly, only shows the relative change of hemodynamics associated with cerebral metabolism during anesthesia. However, it can be used as a complimentary device to monitor DOA especially when EEG provides erroneous DOA estimates with abnormal EEG. Future studies are needed to compare the performance of NIRS in evaluating DOA with index of NIRS combined with conventional methods.

#### 4. Conclusion

NIRS was employed together with LFP during isoflurane inhalation to monitor the DOA in rats, and the results showed that [OHb], [RHb], and [THb] were linearly responded to the level of isoflurane concentration. The reflectance ratio, RRI, between 730nm and 850nm was calculated to minimize the effect of CBV during anesthesia and it was also well corresponded with the change of isoflurane concentration. Therefore, combining NIRS with EEG may provide a new anesthetic depth index in clinical settings.

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